Ochratoxin A ELISA test kit

INSTRUCTION MANUAL

FOR PRODUCT No: LT43001AYSL

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MANUAL VERSION 1.01

INTRODUCTION

Ochratoxin A are secondary metabolites of Aspergillus and Penicillium strains, exist widely in grain and grain products, spices and coffee beans and other products. Ochratoxin A has a strong liver and kidney toxicity, and has teratogenic, mutagenic and carcinogenic effects, which is serious harmful to human health.

Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) has been a common method of Ochratoxin A detection, but sample preparation and samples analysis of this method is time-consuming and exhausting. The use of Ochratoxin A ELISA kit to analyze Ochratoxin A residue of samples is fast and accurate. This kit is a new generation of fungal toxins detection products in application of ELISA technology, to minimize operating errors and work intensity.

PRINCIPLE

This test kit is based on the competitive enzyme immunoassay for the detection of Ochratoxin A in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Ochratoxin A in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti- Ochratoxin A antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Ochratoxin A in it. This value is compared to the standard curve and the Ochratoxin A concentration is subsequently obtained.

MATERIALS SUPPLIED WITH THIS KIT

1	Micro-well strips	12 strips with 8 removable wells each	5	Stop solution	10 ml
2	OTA 5 standards (2 ml each)	0ppb, 0.2ppb, 0.5ppb, 2ppb, 4ppb	6	10X concentrated wash buffer	50 ml
3	OTA Enzyme conjugate	7 ml	7	Sample extract	1 bottle
4	Substrate	12 ml			

MATERIALS REQUIRED BUT NOT PROVIDED

1) Micropipettors and disposable tips: 0.5µl-10µl, 10µl-100µl, 100µl-1000µl.

2) 37 ℃ Incubator.

3) Measuring cylinder: 500 ml.

4) 96 wells microplate reader.

5) Distilled/De-ionized water.

6) Microplate Washer.

TECHNICAL SPECIFICATIONS

Sensitivity: 0.2 ppb

Incubator temperature: 25℃

Incubator time: 15min-30min

Detection limit:

Grain, Feed -2.5ppb

Cross-reaction rate:

Ochratoxin A - 100%

Ochratoxin B - 43%

Ochratoxin C - 5%

Recovery rate:

Feed, Rice - 90±25%

Corn, Peanut - 85±25%

SAMPLE PRE-TREATMENT

Solution preparation before sample pre-treatment:

1) Washing buffer

Use 1 part of concentrated wash buffer (10×) and dissolve with 9 parts of deionized water to obtain the ready to use washing buffer. It is stable for 1 month when stored at 4 ℃.

2) Sample extract solution

Dissolve Sample extract reagent into 1L distilled water completely.

Preparation of Grain, Feed

1) Weigh 0.25g crushed sample, add 10ml Sample extract solution, shake violently for 3min;

2) Centrifuge at 3500 g for 10min;

3) Take 100ul up-layer clear liquid to test.(or filter by filter paper, take filtrate)

Dilution factor: 40

ELISA PROCEDURES

Instructions

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken evenly before use;

2. Put the required micro-well strips into plate frames. Re-seal the unused microplate, store at 2-8 ℃, not frozen.

3. Washing buffer also need to return to room temperature before use.

4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.

5. Add standard/sample: Add 50 µl of the sample or the standard solution into separate duplicate wells, then add OTA Enzyme Conjugate, 50µl/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, **incubate at 25℃ for 30 min in the dark.**

6. Wash microplate: Carefully open the cover membrance, pour liquid out of microwell; add 300 µl/well of washing buffer, wash fully for 5 times, 30 s each time, then take out and flap to dry with absorbent paper.(Use unused spear to pierce bubble after dry)

7. Coloration: add 100 µl of substrate into each well. Mix gently by shaking the plate manually, and **incubate at 25℃ for 30 min in the dark for coloration.**

8. Determination: add 50 µl of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value of every well.

RESULTS JUDGEMENT

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Aflatoxin B1 in the sample

QUALITATIVE DETERMINATION

The concentration range (ppb) can be obtained by compared the average absorbance value with standards. Suppose absorbance value of

Sample One is 0.659, Sample Two is 1.525, and the standards are: 0ppb of 2.101, 0.2ppb of 1.738, 0.5ppb of 1.313, 2ppb of 0.831, 4ppb of 0.262. Then the concentration of the sample one is in the range of 2ppb - 4ppb, Sample Two is 0.2ppb - 0.5ppb. The concentration range of ochratoxin A in the samples can be obtained by multiplied by the corresponding dilution of the sample.

QUANTITATIVE ANALYSIS

In order to calculate the concentration of samples, a standard curve should be made. Before standard curve is made, the concept of % absorbance should be known.

Calculation of % absorbance:

Percentage of absorbance value = $B/B_0 \times 100\%$

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0 ng/mL standard solution

The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the ochratoxin A concentration (µg/kg). The ochratoxin A concentration in µg/kg (ppb) corresponding to the absorbance of each sample can be read from the calibration curve.

PRECAUTIONS

1. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.

2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable

reproducibility; so continue to next step immediately after washing.

3. Mix evenly before adding any reagents.

4. The stop solution is the 0.5 M sulfuric acid solution, avoid contacting with the skin.

5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.

6. Storage: store at 2-8 ℃, not frozen. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.

7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value (450/630nm) of the 0 standard solution (0 ppb) of less than 0.5 (A450nm<0.5) indicates its degeneration.

8. After adding the substrate, the general color rendering time can be 15 ~ 30min. If the color is lighter, extended reaction time to 35min (or longer), do not beyond 40min. On the contrary, shorten reaction time.

9. The optimum reaction temperature is **25 ℃**, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

STORAGE AND EXPIRY DATE

Storage: store at 2-8 ℃, not frozen.

Expiry date: 12 months; date of production is on box.

Note: If the Vacuum package of microplate has leakage, it is still valid to use, do not affect the test result, be relax to use.